Apical junctional complexes and cell polarity

Q Wang[1] and B Margolis[1]

[1]Department of Internal Medicine and Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan, USA

Recent studies have greatly expanded our knowledge of initial events that lead to epithelial cell polarity. Epithelial polarity is defined, in part, by apical cell–cell tight junctions that separate the plasma membrane into the apical domain and the basolateral domain, as well as the zonula adherens that mediate intercellular adhesion. The process of epithelial polarization is closely coupled to the biogenesis of these junctions. Studies in mammalian epithelial cells and lower organisms have identified two evolutionarily conserved junctional complexes as important epithelia polarity regulators: the Crumbs complex and the partitioning defective complex. Disruption of the components of the two complexes leads to a disorder of epithelial cell polarity and defects in junction formation or maintenance. Recent discoveries have revealed more details of how the two junctional polarity complexes function to establish epithelial polarity. They also raised the question about the relationship between polarity and adhesion. Although it is widely accepted that cell–cell adhesion provides a landmark from which polarity can proceed, there are results pointing to the possibility that polarity complexes can regulate cell–cell adhesion. It seems likely that proteins that control cell adhesion and cell polarity work intimately together to establish final epithelial polarity.

Kidney International (2007) **72,** 1448–1458; doi:10.1038/sj.ki.5002579; published online 3 October 2007

KEYWORDS: epithelial polarity; tight junctions; adherens junctions; CRB complex; PAR complex; EMT

Correspondence: *B Margolis, Department of Internal Medicine and Biological Chemistry, University of Michigan Medical School, Room 1528 BSRB, 109 Zina Pitcher Place, Ann Arbor, Michigan 48109-2200, USA. E-mail: bmargoli@umich.edu*

Received 19 June 2007; revised 17 August 2007; accepted 21 August 2007; published online 3 October 2007

The bodies of Metazoa enclose numerous highly organized cavities and compartments that are lined by sheets of epithelial cells. To protect the integrity of these cavities and compartments, epithelial cells have developed various intercellular junctions so that they are tightly packed and strongly adherent to one another. These junctions include the tight junctions (TJs) and the zonula adherens (ZA), which together comprise the apical junctional complexes. In addition to the protection function, epithelial cells are highly polarized and they mediate diverse polarized activities including absorption, secretion, transcellular transport, and sensation. The polarization of epithelial cells is reflected by the asymmetric distribution of proteins and lipids into the apical and basolateral surfaces. The apical domain faces the lumen while the basolateral domain consists of the basal domain that contacts the basement membrane and the lateral domain that contacts the neighboring cells. The process of apical–basal polarization is closely coupled to the establishment of the apical junctional complexes.

The TJ, also referred to as the zonula occludens, is the apical most structure of the intercellular junctional complex. It carries out two important functions: first, it forms tight seals between epithelial cells and creates a selectively permeable barrier to diffusion through the intercellular space, namely the barrier function;[1] second, it physically separates the apical and basolateral membranes and prevents the intermixing of the components of the two membrane domains, namely the fence function.[2] TJs are revealed to be the tight apposition of neighboring epithelial cells in conventional electron micrographs, while in freeze-fracture electron micrographs, they appear as a continuous network of parallel and interconnected strands that circumscribe the apex of lateral membranes.[3] TJs are composed of three families of transmembrane proteins: occludin, claudins, and junctional adhesion molecules. They reach across the intercellular space and connect the membranes of adjacent epithelial cells (reviewed in Shin *et al.*[4]). The functional equivalent structure in *Drosophila* epithelia is the septate junction, which lies basal to the ZA and has a different molecular composition.[5]

The adhesion between epithelial cells is primarily contributed by the ZA, which is also called the adherens junction in vertebrates. It is an adhesive belt that encircles the cell just below the apical surface, and it lays basal to TJs in mammalian epithelial cells. Cadherins represent the primary structural component of ZA and their calcium-dependent

trans-dimerization provides the adhesion between neighboring epithelial cells. Cryo-electron microscopy of the adherens junction reveals rod-like structures extending from the extracellular surface into the intercellular space, and it is suggested that they represent the extracellular domains of E-cadherin.[6] Other adherens junction transmembrane components include Nectins and nectin-like molecules, and they *trans*-interact in a calcium-independent manner.[7]

The apical junctional complexes are dynamic structures. They undergo dramatic rearrangement and redistribution during embryonic development. The cytoplasmic domains of the junctional structural components are associated with various adaptor proteins as well as signaling elements, and they are linked to the cytoskeleton. These connections integrate the dynamics of cell–cell junctions with a number of cellular processes such as migration, proliferation, differentiation as well as pathological processes that include tumor cell metastasis, infiltration, and microbial infections.

APICAL POLARITY COMPLEXES

The formation of junctional complexes is intimately linked to cell polarization. Recent studies in mammalian systems and lower organisms have revealed several evolutionarily conserved protein complexes that regulate cell polarization. The complicated interplay among these complexes and their orderly function regulate the establishment of epithelial cell polarity and the cell–cell junctions. Studies of the apical membrane domain have focused on two major complexes, the Crumbs (CRB) complex and the partitioning defective (PAR) complex.[8] These complexes are important in recognizing the initial polarization cues, and they play a pivotal role in regulating the establishment of apical junctional complexes.

Work in both the mammalian and *Drosophila* systems have demonstrated that the CRB complex and the PAR complex have a conserved function in the establishment and maintenance of apical–basal polarity. In this review, the composition and function of these complexes will be summarized, with an emphasis on recent literature that highlight novel aspects of their structure and function.

CRB complex

The CRB complex is composed of three proteins: CRB, protein associated with Lin Seven 1 (PALS1), and PALS1-associated tight junction protein (PATJ) (see Figure 1). In *Drosophila*, CRB is localized to the apical membrane and the subapical region. The subapical region represents a spot where the apical membrane ends and the lateral membrane begins. In mammalian cells, this is the site of the TJ, but in *Drosophila* no junction is localized at this point. In *Drosophila*, CRB is an important apical membrane determinant, as the plasma membrane-associated expression of CRB is necessary and sufficient to confer apical character on a membrane domain, and overexpression of CRB results in an expansion of the apical plasma membrane with concomitant reduction of the basolateral domain.[9] *Drosophila* CRB is a transmembrane protein with 30 EGF-like and 4 laminin A G-domain-like repeats in its extracellular domain. The exact function of this large extracellular domain is not clear, since a truncated form of CRB devoid of the entire extracellular domain is sufficient to rescue the CRB mutant *Drosophila* embryo.[9] The short cytoplasmic domain of CRB contains two functionally important motifs.[10] The 4.1/ezrin/radaxin/moesin (FERM) domain-binding motif of zebrafish CRB binds an FERM protein Moe, and it has been shown recently that Yurt, the *Drosophila* ortholog of zebrafish Moe, interacts with the *Drosophila* CRB FERM-binding motif.[11,12] This interaction is conserved between the mammalian Yurt orthologs YMO1 and EHM2 and the mammalian CRB proteins, and it may be part of a negative feedback loop that regulates CRB activity.[12] The C-terminal postsynaptic density/discs large/zonula occludens (PDZ) domain-binding motif, on the other hand, is recognized by the PDZ domain of Stardust, the *Drosophila* homolog of PALS1.[13,14] The CRB–Stardust interaction is important for the biogenesis of the ZA, which is a pivotal step in the establishment of epithelial integrity.[15,16]

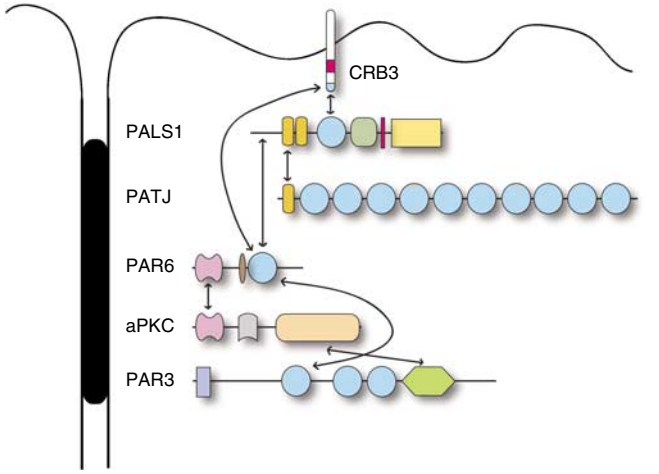

Figure 1 | Domain structures of components of the CRB complex and the PAR complex. Protein domains are represented by filled shapes. Note that CRB3 is depicted larger in proportion to other proteins, and the red and blue fills represent the FERM-binding motif and the PDZ-binding motif respectively. Protein–protein interactions are indicated by double-headed arrows.

Three mammalian CRB proteins have been identified, all of which consist of a transmembrane domain and an intracellular domain with the conserved FERM- and PDZ-binding motifs. CRB1 is the human ortholog of *Drosophila* CRB, and it is expressed primarily in the eye and brain. Mutations in CRB1 cause various diseases including Leber congenital amaurosis and retinitis pigmentosa.[17,18] CRB2 has not been extensively characterized to date. CRB3 is expressed ubiquitously in epithelial tissues, and unlike *Drosophila* CRB and the other two mammalian CRB proteins has a very short extracellular domain. CRB3 is localized to the apical membrane of mammalian epithelial cells and concentrated to TJs, where it interacts with PALS1 with its C-terminal

PDZ-binding motif.[19,20] Overexpression of CRB3 in Madin–Darby canine kidney (MDCK) cells leads to delayed TJ formation and a disruption of cell polarity.[21,22] Introducing CRB3 into the mammary epithelial MCF10A cells, which express little endogenous CRB3, induces the formation of TJs.[23] CRB3 has also been shown to localize to the primary cilia and it is required for ciliogenesis of MDCK cells.[24]

PALS1 is a membrane-associated guanylate kinase protein. It consists of two L27 domains, a PDZ domain, an SH3 domain, a band 4.1-binding domain, and a GUK domain.[25] The two L27 domains mediate its interaction with PATJ and Lin-7, respectively,[19,25] and the PDZ domain binds CRB3 in mammalian epithelial cells.[19,20] The function of the C-terminal SH3, band 4.1-binding, and GUK domains is not known, but a recent report shows that they are an essential part of the PALS1 protein.[26] In addition to these domains, the N-terminal U1 region of both PALS1 and Stardust binds PAR6 (see below).[27,28] RNA interference-mediated inhibition of PALS1 expression in mammalian epithelial cells leads to severe defects in cell–cell junction formation and cell polarity.[26,29] Stardust, the *Drosophila* ortholog of PALS1, acts downstream of CRB to regulate the formation of the ZA and epithelial morphogenesis in flies, and the mutations in Stardust produce a phenotype nearly identical to that of the CRB mutant.[30] The PALS1 ortholog in zebrafish is Nagie Oko. It is essential in the biogenesis of photoreceptor cells in the retina,[31] and it is required for myocardial coherence and heart tube elongation in concert with Heart and Soul/PKCτ.[32] A recent report suggests that Na+,K+-ATPase acts in the same genetic pathway as Nagie Oko in cardiac morphogenesis.[33]

PATJ, the third member of the CRB complex, contains 1 L27 domain at the N terminus and 10 PDZ domains. It interacts with PALS1 through L27 domain dimerization,[19,34] and this interaction is important for the stability of PATJ in mammalian epithelial cells.[26,29] PATJ serves as a scaffold and its multiple PDZ domains interact with various junction structural components, peripheral proteins, and signaling elements, which include claudin-1, zonula occludens-3, and angiomotin.[35,36] Knockdown of PATJ in MDCK cells leads to a delay in TJ formation and cell polarity defects,[37] and the adenovirus protein E4-ORF1 induces the disassembly of TJs by interacting with PATJ and sequestering it from the junctions.[38] *Drosophila* PATJ (DmPATJ), on the other hand, is a much smaller protein with only four PDZ domains besides the L27 domain. DmPATJ stabilizes the CRB complex and is required for rhabdomere stalk membrane maintenance during photoreceptor development.[39] Moreover, DmPATJ has been shown to interact with Frizzled, and it recruits atypical protein kinase C (aPKC) to Frizzled, resulting in the inhibition of Frizzled activity.[40] This study indicates that DmPATJ could be a linker between the apical–basal polarity pathway and the planar cell polarity pathway.

Recent work indicates that PATJ plays a role in regulating the exocytosis of CRB3. Michel *et al.*[41] reported that knockdown of PATJ in Caco2 cells causes the mislocalization of CRB3. CRB3 accumulates in a subapical compartment, and the CRB3-positive compartment partially overlaps with early endosomes indicated by EEA1 staining. Our group observed similar CRB3 retention in PATJ-depleted MDCK cells, and the defect can be rescued when exogenous PATJ is re-introduced (unpublished data). These results suggest that PATJ can control the formation of the apical membrane by regulating CRB3 exocytosis. It was also recently reported that a *Drosophila* syntaxin mutant leads to expansion of the apical membrane similar to that of CRB overexpression, presumably because defective endocytosis leads to excessive CRB on the apical membrane.[42] Therefore, it appears that a balance between exocytosis and endocytosis of CRB is critical for the proper maintenance of the apical membrane domain.

PAR complex

The six *par* genes and *protein kinase C3* were uncovered in a screen for defects in zygotic-axis specification in *Caenorhabditis elegans*.[43] The *par* genes encode primarily scaffolding proteins and serine–threonine kinases.[44] PAR3 and PAR6, two scaffolding proteins as well as atypical PKC constitute the apical polarity PAR complex.

PAR3, PAR6, and aPKC physically interact in a complex fashion (see Figure 1). The aPKC-binding domain of PAR3 directly binds to the kinase domain of aPKC,[45] the PAR3–PAR6 interaction is between the PDZ domain of PAR6 and one of the three PDZ domains of PAR3, and the PB1 domain dimerization mediates the PAR6–aPKC interaction.[46,47] PAR3 can also oligomerize through its N terminus.[48,49] In mammals, there are at least three splice variants of PAR3, four isoforms of PAR6, and two isoforms of aPKC, adding to the complexity.[50–52] The *Drosophila* ortholog of PAR3 is Bazooka (Baz), which directly binds *Drosophila* aPKC and PAR6.[53,54] The small GTPase Cdc42 has been known to be a central cell polarity regulator in many contexts, and the discovery of PAR6 as its effector largely explains this role. PAR6 binds Cdc42-GTP through its semi-Cdc42/Rac interacting binding domain in concert with a part of the PDZ domain.[46,55–57] The involvement of the PDZ domain was elucidated by the crystal structure of PAR6 bound to Cdc42-GTP, which showed that the semi-Cdc42/Rac interacting binding domain and the adjacent PDZ domain form a continuous eight-stranded sheet that binds Cdc42.[58]

In *Drosophila*, the three components of the PAR complex are dependent upon one another for correct localization during epithelial morphogenesis.[53,54,59] Yet, the three proteins do not always colocalize, and very often, PAR3 segregates from PAR6 and aPKC. This phenomenon has been observed in various cell types including the *C. elegans* one-cell embryos,[45] migrating mammalian astrocytes,[60] *Drosophila* photoreceptors,[61] and polarized MDCK cells.[62] These findings suggest that PAR3 and PAR6–aPKC can function independently in many situations.

Compared to the CRB complex, the PAR complex is involved in a broader range of cell types and it regulates more diverse polarity-related cellular events. Besides its established

role in the development of *Drosophila* embryonic ectoderm[53,59] and the formation of TJs in mammalian epithelial cells,[49,63,64] the PAR complex also plays a role in a variety of processes, which include the anterior–posterior axis specification of the *C. elegans* zygote and the *Drosophila* oocyte, the asymmetric division of *Drosophila* neuroblasts and sensory-organ precursor cells, the axon specification of mammalian hippocampal neurons, and the oriented migration and the localization of microtubule-organizing center in various mammalian cell types (reviewed in Macara[44] and Suzuki and Ohno[65]). A common theme is that, the PAR3–PAR6—aPKC complex resides at the side of the cell that develops into the apical/anterior domain, and the PAR1 kinase occupies the opposite side and specifies the basal/posterior domain. The molecular basis of this mutually exclusive localization is beginning to be revealed. In *Drosophila*, PAR1 phosphorylates Baz creating a binding site for the PAR5 protein, a 14-3-3 family member. The subsequent binding of 14-3-3 to Baz inhibits the interaction between Baz and aPKC as well as the formation of the Baz–PAR6–aPKC complex, excluding the complex from the lateral membrane where PAR1 is localized.[66] In mammals, an opposite mechanism has been demonstrated: aPKC phosphorylates PAR1 at the TJs, and the phosphorylation-dependent binding of 14-3-3 to PAR1 dissociates it from the apical domain.[67,68] Nonetheless, 14-3-3 also interacts with phosphorylated PAR3 in mammalian cells and the disruption of this interaction leads to polarity defects,[69] demonstrating cooperative apical and basolateral exclusion mechanisms.

The molecular actions of the PAR complex in epithelial polarity are still being elucidated, but the following model can be proposed as a starting point based on the data from multiple organisms. PAR3 is localized to the cell–cell contact sites early in polarizing cells through its interaction with the TJ structural component junctional adhesion molecule-1 and the adherens junction components Nectin-1 and -3.[70–72] On the other hand, PAR6 and aPKC forms a precomplex with lethal giant larvae (Lgl), which was originally identified as a tumor suppressor gene and later shown to be an important basolateral determinant.[73,74] Upon the binding of Cdc42-GTP, PAR6 undergoes a conformational change and this change results in stronger PAR6–aPKC interaction and higher aPKC kinase activity,[58] leading to the phosphorylation of Lgl. Phosphorylated Lgl dissociates from PAR6–aPKC and frees the interaction interface of PAR6 so that PAR6–aPKC is recruited by PAR3 and forms the PAR3–PAR6–aPKC complex. This in turn prevents the colocalization of Lgl with the PAR complex and limits it to the basolateral domain.[75–77] As a result, the balance between the apical domain and basolateral domain is determined by the activity of the PAR complex at the apical domain and that of Lgl at the lateral domain.[4]

Interaction between the CRB and PAR complexes

The CRB complex and the PAR complex work coordinately to define the apical domain of epithelial cells. The two complexes are mutually dependent upon one another for proper localization in the *Drosophila* photoreceptor,[78] and the knockdown of PALS1 in MDCK cells leads to the mislocalization of PAR3.[29] Biochemical studies have revealed physical interactions between the two complexes (Figure 1). Mammalian PALS1 and PAR6 interact directly through the N terminus of PALS1 and the PDZ domain of PAR6.[27] PDZ domains are typically selective for C-terminal ligands, but non-C-terminal, 'internal' ligands can also be recognized when they are presented in a proper conformation. The PALS1–PAR6 interaction is representative of internal-ligand binding. A PDZ-binding site was identified in the N-terminal U1 region of PALS1, and this site is conserved in Stardust, which binds *Drosophila* PAR6 in a similar manner.[28] There is disagreement on whether the N-terminal L27 domain of PALS1 is involved in presenting the PDZ-binding site.[28,79] The PDZ domain of PAR6 also interacts directly with CRB3,[21] and a recent study found that *Drosophila* CRB binds the PDZ domain of Stardust and *Drosophila* PAR6 with a similar affinity.[80] DmPATJ and *Drosophila* PAR6 interact in *Drosophila* photoreceptors, further tying together these two complexes that are colocalized in the rhabdomere stalk.[61] Besides the above-mentioned interactions that are important for the localization of the two complexes, the aPKC–CRB and aPKC–PATJ interactions in *Drosophila* are functionally significant, since CRB was shown to be an aPKC substrate. A non-phosphorylatable CRB mutant behaves *in vivo* in a dominant-negative fashion and disrupts epithelial cell polarity. Overexpression of a kinase-dead aPKC also causes serious defects in the structure of the epithelial layer, supporting the idea that aPKC-mediated phosphorylation of CRB may be important for cell polarity.[81]

FROM ADHESION TO POLARITY: THE CLASSICAL MODEL

Epithelial cells undergo a dynamic process to reach their fully polarized state. The junctional complexes play a complicated role in polarization because on the one hand they are believed to drive the process, but on the other the formation of mature apical junctional complexes is often used as a benchmark of completed polarization. A great amount of effort has been invested to dissect the molecular events during epithelial cell polarization, and a central question is the nature of the initial polarity cue. It is generally believed that cell–cell contacts and adhesion initiate the polarization of epithelial cells and formation of preliminary junctions precedes the subsequent polarization steps. This view is widely accepted as the classical model of epithelial polarization.

The primary cell–cell adhesive molecule E-cadherin *trans*-interacts in a Ca^{2+}-dependent manner. People have taken advantage of this feature and developed the calcium depletion/repletion (switch) model to study the polarization of mammalian epithelial cells in culture. When cultured in low Ca^{2+} medium, cells round up and lose most contacts with their neighboring cells. At this time, the majority of E-cadherin has been endocytosed and stored in intracellular compartments but there is still a fraction of E-cadherin as

well as other cell adhesion molecules on the cell surface. Upon the addition of Ca^{2+}, E-cadherin from adjacent cells make contact and start the *trans*-dimerization; Nectin and Nectin-like molecules also participate in this initial contact, although it is not clear whether their Ca^{2+}-independent adhesion temporally proceeds that of E-cadherin. This initial contact serves as a spatial cue of apical–basal polarity, and it initiates a series of signaling events and leads to the rapid exocytosis of more E-cadherin and other cell adhesion molecules, including future TJ components. They also form the nascent junctional structures—spot-like adherens junctions at the cell–cell contact sites. The spot-like adherens junctions serve to anchor and nucleate cytoskeletal actin and induce an overall change in cell shape. In the next phase, the TJ components separate from the adherens junction components and move apically to form TJs, and the spot-like adherens junctions fuse into mature adherens junction belts. At the same time, intracellular vesicles dock and fuse to the boundary of the apical and the basolateral membrane domains, which is where the TJs assemble; the addition of lipids and proteins leads to the growth of the lateral membrane and the vertical extension of the epithelial cells (reviewed in Nakanishi and Takai,[7] Suzuki and Ohno,[65] Yeaman *et al.*,[82] and Nelson[83]).

The CRB complex and the PAR complex have been shown to regulate various aspects of this epithelial polarization process. As mentioned above, activation of the PAR complex and the correct localization of the CRB complex are essential for apical membrane determination. Knockdown of PAR3 and the CRB complex proteins leads to the delay or complete disruption of TJ biogenesis. Recent studies of polarity signaling events point increasingly to the role of the apical polarity complexes. Here are highlights of some of the most recent findings.

Asymmetric distribution of phosphoinositides

Cell polarity is defined as the asymmetric distribution of macromolecules, which include not only proteins but also lipids. The polarized localization of phosphoinositides, chiefly phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) and phosphatidylinositol 3,4,5-trisphosphate ($PI(3,4,5)P_3$), is particularly of interest, because $PI(4,5)P_2$ and $PI(3,4,5)P_3$ specifically bind certain protein domains, and they have an important regulatory role in actin cytoskeleton dynamics (reviewed in Yin and Janmey[84]). It has been known that $PI(4,5)P_2$ and $PI(3,4,5)P_3$ are involved in regulating cell migration and cytokinesis, with $PI(3,4,5)P_3$ enriched at the front or at poles, whereas $PI(4,5)P_2$ localized to the back or the furrow.[85] $PI(4,5)P_2$ is converted into $PI(3,4,5)P_3$ by PI3 kinase (PI3K). The coupling of the PI3K pathway and the PAR complex has been reported.[86] aPKC can be directly activated by 3-phosphoinositide-dependent protein kinase-1 in a PI3K activity-dependent manner,[87,88] and the axonally localized PI3K activity is required for proper distribution of PAR3 in hippocampal neurons.[86] However, how $PI(4,5)P_2$ and $PI(3,4,5)P_3$ are involved in epithelial polarity and how they interact with the apical polarity complexes are not known. Several recent studies have shed light on these questions.

Pinal *et al.*[89] studied the *Drosophila* photoreceptors and found that $PI(3,4,5)P_3$ is accumulated within the apical membrane domain while $PI(4,5)P_2$ is enriched at the ZA. This differential distribution is thought to be induced by the localization of PTEN, a lipid phosphatase that converts $PI(3,4,5)P_3$ into $PI(4,5)P_2$. PTEN is localized to photoreceptor ZA, and this localization is dependent on Baz, as the ectopically expressed Baz can recruit PTEN while the ZA localization of PTEN is abolished in *baz* mutant ommatidia.

Two reports from the Mostov group, however, suggest that in mammalian epithelial cells, $PI(4,5)P_2$ and $PI(3,4,5)P_3$ accumulate on the apical membrane and the basolateral membrane respectively.[90,91] Intriguingly, the application of exogenous $PI(3,4,5)P_3$ to the apical side of cells induces apical protrusions within 5 min, and proteins normally localized to the basolateral side are found in those protrusions. In contrast, adding exogenous $PI(4,5)P_2$ to the basal side causes the translocation of apical and TJ proteins to the basal membranes. These results suggest that phosphoinositides are important to specify the identity of the apical and basolateral membrane domains. Mostov and coworkers further showed that the $PI(4,5)P_2$-binding protein annexin2 binds Cdc42, which in turn targets aPKC to the apical membrane.

These reports uncover a new mode of action for the PAR complex in cell polarity regulation, yet important questions remain. A fundamental issue is the causal relationship between the PAR complex and phosphoinositides, for example whether the asymmetric localization of the PAR complex leads to the differential distribution of phosphoinositides, or vice versa. The work in *Drosophila* photoreceptors suggests the former possibility, while work in mammalian epithelial cells indicates the latter. It is also not clear how PTEN is localized to the apical membrane in mammalian epithelial cells as observed in Martin-Belmonte *et al.* Moreover, $PI(3,4,5)P_3$ accumulates on the apical membrane in *Drosophila* photoreceptors while it is on the basolateral membrane domain of mammalian epithelial cells. Is this discrepancy organism-dependent or is it because the photoreceptors are specialized epithelia? Further investigations in other epithelial models will be needed to answer this question.

Local activation/inactivation of small GTPases

The signaling events following the initial polarity cue are of great interest to scientists, because they are the key to understanding how the polarization process is programmed and regulated. Investigators have paid close attention to the Rho family of small GTPases, for example Rho, Rac, and Cdc42, because the engagement of E-cadherin and Nectins triggers the activation of Rac and Cdc42,[7,92] while the level of active Rho is reduced during polarization.[93] In addition, there is an evolutionarily conserved role for these GTPases in

polarity determination (reviewed in Pruyne *et al.*[94] and Garcia *et al.*[95]). It has been suggested that in epithelial cells, Rac and Cdc42 are downstream of the PI3K pathway.[96] However, there is also evidence showing that Rac can be activated by $PI(3,4,5)P_3$ and activated Rac can in turn activate PI3K, amplifying the signal through a positive feedback loop.[97] Recent research has unveiled a common theme in the regulation of the small GTPases: recruitment of GTP exchange factors (GEFs) or GTPase-activating proteins to the cell–cell contact sites so that the activity of small GTPases can be controlled locally. The CRB complex and the PAR complex have both been shown to participate in this process.

Studies have found that PAR6 is a binding partner of a small GTPase GEF, ECT2.[98] Coexpression of PAR6 and ECT2 activates Cdc42 *in vivo*, and ECT2 can increase the kinase activity of aPKC. ECT2 is localized to cell–cell contacts as well as the nucleus. Interestingly, the expression of ECT2 is repressed in low Ca^{2+} medium and restored when Ca^{2+} is replenished.[98] It is possible that the Ca^{2+}-responsive expression of ECT2 is part of the internal force to drive the repolarization of mammalian epithelial cells in the Ca^{2+} switch model. And as PAR6 is itself a Cdc42 effector, the complex of PAR6–Cdc42–ECT2 may represent a positive feedback mechanism.

In 2005, three groups reported the interaction between PAR3 and the Rac GEF T-lymphoma invasion and metastasis (Tiam1/STEF) protein.[99–101] Tiam1 had been previously found to be required for the establishment and maintenance of cadherin-based adhesions,[102] and the discovery of its interaction with PAR3 helped explain this involvement. However, the three studies were carried out in different cell types and they reached different conclusions. Chen and Macara did the study in MDCK cells and found that PAR3 binds to and inhibits Tiam1-mediated Rac activation, resulting in the promotion of TJ assembly without effect on adherens junction formation. In contrast, Mertens *et al.* reported that Tiam1 interacts with PAR3 and aPKC through the activation of Rac in keratinocytes and positively regulates TJ formation in a Cdc42-independent manner. The third study performed in neuroblastoma cells by Nishimura *et al.* proposed that the PAR complex mediates Cdc42-induced Rac activation via STEF/Tiam1, and that this process is required for the establishment of neuronal polarity. These conflicting results may be a result of the different cell models used, but all indicate a new role for the PAR complex, in regulating actin dynamics. This new role is further demonstrated by a more recent paper from Chen and Macara, showing that PAR3 binds to and inhibits the kinase activity of LIM kinase 2 and thus suppresses coffilin phosphorylation and its actin-severing activity.[103]

A recent paper from the Pawson group discovered that a Cdc42-specific GTPase-activating protein Rich1 binds PATJ through a scaffold protein angiomotin.[36] A point mutation in the GTPase-activating protein domain, or depletion of Rich1 by RNA interference, causes a profound defect in TJs, and the phenotype is very similar to the effect of Rab13 mutants, a regulator of the recycling of TJ components. This observation suggests that Rich1 might play a role in maintenance rather than in the initial establishment of junctions, by regulating the recycling of TJ components.[104] It also suggests that the CRB complex and the PAR complex, which is also found associated with Rich1/angiomotin, might be involved in the initial sorting and/or the recycling of TJ proteins, and are therefore important for both the establishment and the maintenance of apical junctional complexes.

In addition to these reports, the Rho GEF GEF-H1/Lcf has been shown to interact with the TJ-associated protein cingulin to inhibit Rho signaling and G1/S phase transition,[105] and the Cdc42 GEF Tuba has been suggested to bind zonula occludens-1 to maintain proper junction configuration.[106] These data further support a common theme that the local activation/inactivation of Rho family small GTPases is critical in TJ formation and epithelial polarity.

POLARITY BEFORE ADHESION?

Dominant as the adhesion-to-polarity model is, recent studies have indicated the possibility that initiation of polarization could be independent of adhesion. In 2004, Baas *et al.*[107] discovered that activation of the mammalian PAR4 protein LKB1 is sufficient to induce the remodeling of actin cytoskeleton in contact-naïve intestinal epithelial cells to form brush borders, and junctional proteins, zonula occludens-1 and p120, redistribute to a dotted circle at the periphery of single cells. The CRB complex and the PAR complex have also been suggested in a few cases to initiate polarity in the absence of adhesion.

PAR complex in biogenesis of *Drosophila* primary epithelial cells

Drosophila embryos develop their primary epithelial cells through a process called cellularization. After fertilization, the *Drosophila* egg undergoes 13 nuclear divisions without cytokinesis. At the end of the 13th cell cycle, ~5000 nuclei form a monolayer just beneath the egg membrane. Then the egg membrane invaginates into the cytoplasm to surround each nucleus during the 14th cell cycle and establishes an epithelium of highly columnar cells. This process is called cellularization (reviewed in Tepass *et al.*[108]). Cellularization can be further divided into four stages according to the localization of the cadherin–catenin complex, the molecular basis of ZA. First, the original plasma membrane is internalized and transcytosed to form the downward growing furrow canal with the cadherin–catenin complex localized to the tip of the furrow canal at the basal junctions. Second, new protein and membrane materials are exocytosed to the apical side, while the cadherin–catenin complex moves up and forms multiple spot adherens junctions. Then the invagination continues and the spot adherens junctions move upward toward the apicolateral edge of the cells. At the final stage, spot adherens junctions fuse into a circumferential belt to form the ZAs with new material being added below the newly formed ZA. Therefore, the lateral membrane elongates as the

furrow canal is further growing downward (reviewed in Nelson[83] and Tepass *et al.*[108]).

A careful study of the protein localization and movement in the *Drosophila* cellularization model showed that the correct localization of Baz is independent of ZA, while the recruitment of *Drosophila* E-cadherin into apical spot junctions requires Baz.[109] The ZA has essential roles for the proper development of the epidermis, since the absence of ZA results in widespread cell dissociation and depolarization during gastrulation,[109] but ZA or spot adherens junctions are not the first polarity cue, and rather, Baz acts as a primary polarity landmark that positions adherens junctions and aPKC. The PAR complex also provides the internal cue of anterior–posterior polarization in *C. elegans* one-cell embryos. How then is Baz localized to the apical side in the first place? Harris and Peifer[110] followed this question by showing that localization of Baz is a three-fold story. During early cellularization, Baz is positioned by the apical actin and its translocation from the basal side to the apical side is dependent on the microtubule minus end transporter, Dynein. Later in gastrulation, Baz is segregated from PAR6 and aPKC by a yet unknown mechanism, while CRB may be important for this segregation by blocking the Baz–PAR6 and Baz–aPKC interactions.[110]

However, similar observations in mammalian epithelial cells have not been reported. There are several possible explanations. First, mammalian epithelial cells have junctional adhesion molecule-1, Nectin-1, and Nectin-3 to anchor PAR3 to the cell–cell contact sites, but clear orthologs of junctional adhesion molecule and Nectin are absent in *Drosophila*.[65] Second, cellularization in essence is a process of cytokinesis. The initial positioning of Baz might reflect its involvement in cytokinesis. This cellularization process does not occur in mammalian epithelia, which arise from preexisting mesenchymal cells that transit to epithelia.

PALS1 in E-cadherin trafficking

E-cadherin is the major structural component of adherens junctions. As discussed above, the engagement of E-cadherin is likely to initiate the subsequent translocation of cell adhesion molecules and junctional proteins, including the CRB complex and the PAR complex (see above). However, a potential confusing role for PALS1 in E-cadherin trafficking has been revealed recently and adds to the evidence that polarity can control adhesion. Our group found that knockdown of PALS1 in MDCK cells not only causes serious TJ defects, but also disrupts adherens junctions by interfering with E-cadherin trafficking. E-cadherin is retained in intracellular puncta in the cell periphery, and cells fail to make contacts to one another. The exocytosis of E-cadherin is slowed, and the ineffectiveness of E-cadherin cell surface delivery can be partially explained by the mislocalization of the exocyst.[26] These results suggest that the activity of these polarity proteins is not always secondary to cell–cell contacts, but rather involved in the formation of cell adhesion. *Drosophila* CRB and Stardust were known to play an important role in the biogenesis of epithelial ZA, but a similar regulatory role for the CRB complex in adherens junction formation had not been detected in mammalian epithelial cells until this study. This study shows that mammalian PALS1 can regulate E-cadherin exocytosis indicating that the involvement of PALS1 in adherens junction formation is conserved among species. This again points to the question of whether polarity proteins regulate cell adhesion or vice versa. The most likely answer is that processes involving polarity complexes and adhesion complexes work intimately together to establish final epithelial polarity. It seems likely that early adhesion events activate polarity proteins, which then feed forward to reinforce adhesion, which promotes further polarization (summarized in Figure 2).

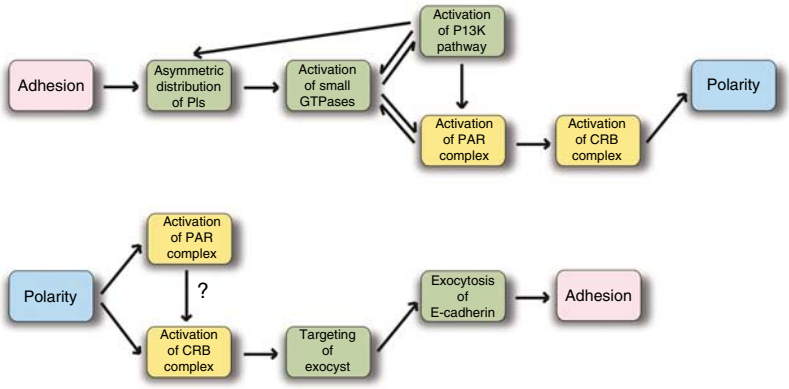

Figure 2 | The classical adhesion-to-polarity model and the polarity-to-adhesion hypothesis. The text lays out two opposing views of polarity determination. In the classic model shown on top, cell–cell adhesion precedes polarity determination. In the second model below, polarity is determined intrinsically and leads to lateral cell–cell adhesion. However, in the later model it is not clear how the activation of PAR complex precedes adhesion. It seems most likely that both pathways are operative with a small amount of adhesion feeding forward through polarity complexes to reinforce adhesion. Activity of the CRB complex and the PAR complex are denoted by yellow shades, and other steps of the polarization process are in green.

APICAL POLARITY COMPLEXES AND EMT

Epithelial polarization is a dynamic process, and the reverse process is also present in physiological and pathological contexts. Epithelial cells can acquire a fibroblastoid phenotype in a process known as epithelial-to-mesenchymal transition, or EMT. EMT is associated with the loss of the apical–basal polarity axis, the dissociation of apical junctional complexes, and a profound change in the protein expression profile. EMT is required for various embryological stages such as gastrulation, neurulation, and neural crest development, and it is correlated with the progression of carcinomas to an invasive and metastatic state.[111] The reverse process mesenchymal-to-epithelial transition (MET) is responsible for the formation of much of the kidney epithelia during development.[112] We are only beginning to understand this process but it seems clear that alterations in transcription factor activity lead to changes in the expression of adhesion, cytoskeletal and polarity proteins that can transform mesenchyme into epithelia. An understanding of EMT and MET can provide clues to the important events that lead to polarization and TJ formation.

Growth factor, transforming growth factor-β (TGFβ), is able to induce EMT and junction dissolution in certain epithelial cell lines,[113] and two recent reports showed that PAR6 plays a key role in this process.[114,115] TGFβ signals through two transmembrane serine–threonine kinases, the type II (TβRII) and type I (TβRI) receptors. TβRII is localized to puncta over the surface of epithelial cells, whereas TβRI is exclusively at TJs associated with occludin. Both reports found that PAR6 interacts with TβRI. Upon TGFβ treatment, TβRII is translocated to TJs and it phosphorylates PAR6 on Ser345. The phosphorylated PAR6 remains bound to the TβR complex and recruits the E3 ubiquitin ligase Smurf1, which in turn ubiquitinates local RhoA and designates it for degradation. In polarized epithelial cells, RhoA contributes to maintain apico–basal polarity and cell–cell junctions by stabilizing cortical actin,[116,117] and the degradation of Rho leads to dissolution of TJs and EMT (reviewed in Bose and Wrana[111]). These findings elucidate the key steps between TGFβ treatment and TJ dissociation, and they indicate that the phosphorylation of PAR6 may be a switch for epithelial cells between the polarized state and EMT.

EMT is associated with a substantial change in gene expression program, and one of the key transcription regulators is Snail, which lies in the converging point of various signaling pathways that can induce EMT, including the TGFβ pathway. Snail can bind to the E-box elements in promoters and repress protein expression. Its primary target is E-cadherin, the adherens junction structural component; Snail also downregulates the expression of TJ components including occludin and claudins (reviewed in Barrallo-Gimeno and Nieto[118]). Our group recently found that the protein level of the CRB complex is reduced during Snail-induced EMT; there are multiple E-boxes in the promoter of CRB3, which makes CRB3 a possible target of Snail (unpublished data). This has been found by another group looking at another transcription factor that induces EMT called zinc-finger E box-binding homeobox (ZEB).[119] Taken together it is clear that factors that promote EMT target both polarity and adhesion complexes and it highlights the likely importance of both complexes in cell polarization.

CONCLUSIONS

In summary, a combination of genetic and biochemical studies have greatly expanded our knowledge of TJ formation and the initiation of epithelial polarity by apical junctional complexes. However, there is still much to be learned about these processes. One only has to look at the process of polarized secretion and budding in yeast models to understand how complex these processes can be and it is certain that mammalian polarization is more complex.[94] There are also distinct types of polarization in the mammalian kidney including planar and apico–basal polarity. Even looking only at apico–basal polarization there are different models that need to be considered. Many groups have studied epithelial polarization after calcium switch. As discussed in the review, this model uses low calcium to disrupt cell adhesion and then studies epithelial polarization as calcium and cell–cell adhesion is restored. This model has merit and has uncovered many important concepts and it may have relevance to the loss of polarization that occurs during ischemic renal injury. However this is not a good model for the polarization of epithelia that occurs as epithelia are formed during development in MET.[112] In the calcium switch model, polarity and adhesion proteins are present and then restored to their place in the polarizing epithelia, whereas in MET a large number of polarity proteins are induced by changes in gene expression. The temporal and spatial generation of polarity in MET is likely to be an extremely complex process, as these new translated polarity combinatorially interact to form a polarized epithelial cell. This will be a fertile field for further study as we attempt to understand the basic details of epithelial cell formation.

REFERENCES

1. Diamond JM. Twenty-first Bowditch lecture. The epithelial junction: bridge, gate, and fence. *Physiologist* 1977; **20**: 10–18.
2. Dragsten PR, Blumenthal R, Handler JS. Membrane asymmetry in epithelia: is the tight junction a barrier to diffusion in the plasma membrane? *Nature* 1981; **294**: 718–722.
3. Claude P. Morphological factors influencing transepithelial permeability: a model for the resistance of the zonula occludens. *J Membr Biol* 1978; **39**: 219–232.
4. Shin K, Fogg VC, Margolis B. Tight junctions and cell polarity. *Annu Rev Cell Dev Biol* 2006; **22**: 207–235.
5. Knust E, Bossinger O. Composition and formation of intercellular junctions in epithelial cells. *Science* 2002; **298**: 1955–1959.
6. Miyaguchi K. Ultrastructure of the zonula adherens revealed by rapid-freeze deep-etching. *J Struct Biol* 2000; **132**: 169–178.
7. Nakanishi H, Takai Y. Roles of nectins in cell adhesion, migration and polarization. *Biol Chem* 2004; **385**: 885–892.
8. Margolis B, Borg JP. Apicobasal polarity complexes. *J Cell Sci* 2005; **118**: 5157–5159.
9. Wodarz A, Hinz U, Engelbert M, Knust E. Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of *Drosophila*. *Cell* 1995; **82**: 67–76.

10. Klebes A, Knust E. A conserved motif in Crumbs is required for E-cadherin localisation and zonula adherens formation in *Drosophila*. *Curr Biol* 2000; **10**: 76–85.
11. Jensen AM, Westerfield M. Zebrafish mosaic eyes is a novel FERM protein required for retinal lamination and retinal pigmented epithelial tight junction formation. *Curr Biol* 2004; **14**: 711–717.
12. Laprise P, Beronja S, Silva-Gagliardi NF *et al.* The FERM protein Yurt is a negative regulatory component of the Crumbs complex that controls epithelial polarity and apical membrane size. *Dev Cell* 2006; **11**: 363–374.
13. Bachmann A, Schneider M, Theilenberg E *et al. Drosophila* Stardust is a partner of Crumbs in the control of epithelial cell polarity. *Nature* 2001; **414**: 638–643.
14. Hong Y, Stronach B, Perrimon N *et al. Drosophila* Stardust interacts with Crumbs to control polarity of epithelia but not neuroblasts. *Nature* 2001; **414**: 634–638.
15. Grawe F, Wodarz A, Lee B *et al.* The *Drosophila* genes crumbs and stardust are involved in the biogenesis of adherens junctions. *Development* 1996; **122**: 951–959.
16. Tepass U. Crumbs, a component of the apical membrane, is required for zonula adherens formation in primary epithelia of *Drosophila*. *Dev Biol* 1996; **177**: 217–225.
17. den Hollander AI, ten Brink JB, de Kok YJ *et al.* Mutations in a human homologue of *Drosophila* crumbs cause retinitis pigmentosa (RP12). *Nat Genet* 1999; **23**: 217–221.
18. den Hollander AI, Heckenlively JR, van den Born LI *et al.* Leber congenital amaurosis and retinitis pigmentosa with Coats-like exudative vasculopathy are associated with mutations in the crumbs homologue 1 (CRB1) gene. *Am J Hum Genet* 2001; **69**: 198–203.
19. Roh MH, Makarova O, Liu CJ *et al.* The Maguk protein, Pals1, functions as an adapter, linking mammalian homologues of Crumbs and Discs Lost. *J Cell Biol* 2002; **157**: 161–172.
20. Makarova O, Roh MH, Liu CJ *et al.* Mammalian Crumbs3 is a small transmembrane protein linked to protein associated with Lin-7 (Pals1). *Gene* 2003; **302**: 21–29.
21. Lemmers C, Michel D, Lane-Guermonprez L *et al.* CRB3 binds directly to Par6 and regulates the morphogenesis of the tight junctions in mammalian epithelial cells. *Mol Biol Cell* 2004; **15**: 1324–1333.
22. Roh MH, Fan S, Liu CJ, Margolis B. The Crumbs3-Pals1 complex participates in the establishment of polarity in mammalian epithelial cells. *J Cell Sci* 2003; **116**: 2895–2906.
23. Fogg VC, Liu CJ, Margolis B. Multiple regions of Crumbs3 are required for tight junction formation in MCF10A cells. *J Cell Sci* 2005; **118**: 2859–2869.
24. Fan S, Hurd TW, Liu CJ *et al.* Polarity proteins control ciliogenesis via kinesin motor interactions. *Curr Biol* 2004; **14**: 1451–1461.
25. Kamberov E, Makarova O, Roh M *et al.* Molecular cloning and characterization of Pals, proteins associated with mLin-7. *J Biol Chem* 2000; **275**: 11425–11431.
26. Wang Q, Chen XW, Margolis B. PALS1 regulates E-cadherin trafficking in mammalian epithelial cells. *Mol Biol Cell* 2007; **18**: 874–885.
27. Hurd TW, Gao L, Roh MH *et al.* Direct interaction of two polarity complexes implicated in epithelial tight junction assembly. *Nat Cell Biol* 2003; **5**: 137–142.
28. Wang Q, Hurd TW, Margolis B. Tight junction protein Par6 interacts with an evolutionarily conserved region in the amino terminus of PALS1/stardust. *J Biol Chem* 2004; **279**: 30715–30721.
29. Straight SW, Shin K, Fogg VC *et al.* Loss of PALS1 expression leads to tight junction and polarity defects. *Mol Biol Cell* 2004; **15**: 1981–1990.
30. Tepass U, Knust E. Crumbs and stardust act in a genetic pathway that controls the organization of epithelia in *Drosophila melanogaster*. *Dev Biol* 1993; **159**: 311–326.
31. Wei X, Malicki J. Nagie Oko, encoding a MAGUK-family protein, is essential for cellular patterning of the retina. *Nat Genet* 2002; **31**: 150–157.
32. Rohr S, Bit-Avragim N, Abdelilah-Seyfried S. Heart and soul/PRKCi and nagie oko/Mpp5 regulate myocardial coherence and remodeling during cardiac morphogenesis. *Development* 2006; **133**: 107–115.
33. Cibrian-Uhalte E, Langenbacher A, Shu X *et al.* Involvement of zebrafish Na+,K+ ATPase in myocardial cell junction maintenance. *J Cell Biol* 2007; **176**: 223–230.
34. Lemmers C, Medina E, Delgrossi MH *et al.* hINADl/PATJ, a homolog of discs lost, interacts with crumbs and localizes to tight junctions in human epithelial cells. *J Biol Chem* 2002; **277**: 25408–25415.
35. Roh MH, Liu CJ, Laurinec S, Margolis B. The carboxyl terminus of zona occludens-3 binds and recruits a mammalian homologue of discs lost to tight junctions. *J Biol Chem* 2002; **277**: 27501–27509.
36. Wells CD, Fawcett JP, Traweger A *et al.* A Rich1/Amot complex regulates the Cdc42 GTPase and apical-polarity proteins in epithelial cells. *Cell* 2006; **125**: 535–548.
37. Shin K, Straight S, Margolis B. PATJ regulates tight junction formation and polarity in mammalian epithelial cells. *J Cell Biol* 2005; **168**: 705–711.
38. Latorre IJ, Roh MH, Frese KK *et al.* Viral oncoprotein-induced mislocalization of select PDZ proteins disrupts tight junctions and causes polarity defects in epithelial cells. *J Cell Sci* 2005; **118**: 4283–4293.
39. Richard M, Grawe F, Knust E. DPATJ plays a role in retinal morphogenesis and protects against light-dependent degeneration of photoreceptor cells in the *Drosophila* eye. *Dev Dyn* 2006; **235**: 895–907.
40. Djiane A, Yogev S, Mlodzik M. The apical determinants aPKC and dPatj regulate Frizzled-dependent planar cell polarity in the *Drosophila* eye. *Cell* 2005; **121**: 621–631.
41. Michel D, Arsanto JP, Massey-Harroche D *et al.* PATJ connects and stabilizes apical and lateral components of tight junctions in human intestinal cells. *J Cell Sci* 2005; **118**: 4049–4057.
42. Lu H, Bilder D. Endocytic control of epithelial polarity and proliferation in *Drosophila*. *Nat Cell Biol* 2005; **7**: 1232–1239.
43. Kemphues KJ, Priess JR, Morton DG, Cheng NS. Identification of genes required for cytoplasmic localization in early *C. elegans* embryos. *Cell* 1988; **52**: 311–320.
44. Macara IG. Parsing the polarity code. *Nat Rev Mol Cell Biol* 2004; **5**: 220–231.
45. Tabuse Y, Izumi Y, Piano F *et al.* Atypical protein kinase C cooperates with PAR-3 to establish embryonic polarity in *Caenorhabditis elegans*. *Development* 1998; **125**: 3607–3614.
46. Qiu RG, Abo A, Steven Martin G. A human homolog of the *C. elegans* polarity determinant Par-6 links Rac and Cdc42 to PKCzeta signaling and cell transformation. *Curr Biol* 2000; **10**: 697–707.
47. Noda Y, Kohjima M, Izaki T *et al.* Molecular recognition in dimerization between PB1 domains. *J Biol Chem* 2003; **278**: 43516–43524.
48. Benton R, St Johnston D. A conserved oligomerization domain in *Drosophila* Bazooka/PAR-3 is important for apical localization and epithelial polarity. *Curr Biol* 2003; **13**: 1330–1334.
49. Mizuno K, Suzuki A, Hirose T *et al.* Self-association of PAR-3-mediated by the conserved N-terminal domain contributes to the development of epithelial tight junctions. *J Biol Chem* 2003; **278**: 31240–31250.
50. Gao L, Macara IG, Joberty G. Multiple splice variants of Par3 and of a novel related gene, Par3L, produce proteins with different binding properties. *Gene* 2002; **294**: 99–107.
51. Gao L, Macara IG. Isoforms of the polarity protein par6 have distinct functions. *J Biol Chem* 2004; **279**: 41557–41562.
52. Yoshii T, Mizuno K, Hirose T *et al.* sPAR-3, a splicing variant of PAR-3, shows cellular localization and an expression pattern different from that of PAR-3 during enterocyte polarization. *Am J Physiol Gastrointest Liver Physiol* 2005; **288**: G564–G570.
53. Wodarz A, Ramrath A, Grimm A, Knust E. *Drosophila* atypical protein kinase C associates with Bazooka and controls polarity of epithelia and neuroblasts. *J Cell Biol* 2000; **150**: 1361–1374.
54. Rolls MM, Albertson R, Shih HP *et al. Drosophila* aPKC regulates cell polarity and cell proliferation in neuroblasts and epithelia. *J Cell Biol* 2003; **163**: 1089–1098.
55. Joberty G, Petersen C, Gao L, Macara IG. The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. *Nat Cell Biol* 2000; **2**: 531–539.
56. Johansson A, Driessens M, Aspenstrom P. The mammalian homologue of the *Caenorhabditis elegans* polarity protein PAR-6 is a binding partner for the Rho GTPases Cdc42 and Rac1. *J Cell Sci* 2000; **113**(Part 18): 3267–3275.
57. Lin D, Edwards AS, Fawcett JP *et al.* A mammalian PAR-3–PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. *Nat Cell Biol* 2000; **2**: 540–547.
58. Garrard SM, Capaldo CT, Gao L *et al.* Structure of Cdc42 in a complex with the GTPase-binding domain of the cell polarity protein, Par6. *EMBO J* 2003; **22**: 1125–1133.
59. Petronczki M, Knoblich JA. DmPAR-6 directs epithelial polarity and asymmetric cell division of neuroblasts in *Drosophila*. *Nat Cell Biol* 2001; **3**: 43–49.
60. Etienne-Manneville S, Hall A. Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. *Cell* 2001; **106**: 489–498.
61. Nam SC, Choi KW. Interaction of Par-6 and Crumbs complexes is essential for photoreceptor morphogenesis in *Drosophila*. *Development* 2003; **130**: 4363–4372.

62. Vogelmann R, Nelson WJ. Fractionation of the epithelial apical junctional complex: reassessment of protein distributions in different substructures. *Mol Biol Cell* 2005; **16**: 701–716.
63. Suzuki A, Yamanaka T, Hirose T *et al.* Atypical protein kinase C is involved in the evolutionarily conserved par protein complex and plays a critical role in establishing epithelia-specific junctional structures. *J Cell Biol* 2001; **152**: 1183–1196.
64. Gao L, Joberty G, Macara IG. Assembly of epithelial tight junctions is negatively regulated by Par6. *Curr Biol* 2002; **12**: 221–225.
65. Suzuki A, Ohno S. The PAR–aPKC system: lessons in polarity. *J Cell Sci* 2006; **119**: 979–987.
66. Benton R, St Johnston D. *Drosophila* PAR-1 and 14-3-3 inhibit Bazooka/PAR-3 to establish complementary cortical domains in polarized cells. *Cell* 2003; **115**: 691–704.
67. Hurov JB, Watkins JL, Piwnica-Worms H. Atypical PKC phosphorylates PAR-1 kinases to regulate localization and activity. *Curr Biol* 2004; **14**: 736–741.
68. Suzuki A, Hirata M, Kamimura K *et al.* aPKC acts upstream of PAR-1b in both the establishment and maintenance of mammalian epithelial polarity. *Curr Biol* 2004; **14**: 1425–1435.
69. Hurd TW, Fan S, Liu CJ *et al.* Phosphorylation-dependent binding of 14-3-3 to the polarity protein Par3 regulates cell polarity in mammalian epithelia. *Curr Biol* 2003; **13**: 2082–2090.
70. Ebnet K, Suzuki A, Horikoshi Y *et al.* The cell polarity protein ASIP/PAR-3 directly associates with junctional adhesion molecule (JAM). *EMBO J* 2001; **20**: 3738–3748.
71. Itoh M, Sasaki H, Furuse M *et al.* Junctional adhesion molecule (JAM) binds to PAR-3: a possible mechanism for the recruitment of PAR-3 to tight junctions. *J Cell Biol* 2001; **154**: 491–497.
72. Takekuni K, Ikeda W, Fujito T *et al.* Direct binding of cell polarity protein PAR-3 to cell–cell adhesion molecule nectin at neuroepithelial cells of developing mouse. *J Biol Chem* 2003; **278**: 5497–5500.
73. Ohshiro T, Yagami T, Zhang C, Matsuzaki F. Role of cortical tumour-suppressor proteins in asymmetric division of *Drosophila* neuroblast. *Nature* 2000; **408**: 593–596.
74. Peng CY, Manning L, Albertson R, Doe CQ. The tumour-suppressor genes lgl and dlg regulate basal protein targeting in *Drosophila* neuroblasts. *Nature* 2000; **408**: 596–600.
75. Betschinger J, Mechtler K, Knoblich JA. The Par complex directs asymmetric cell division by phosphorylating the cytoskeletal protein Lgl. *Nature* 2003; **422**: 326–330.
76. Plant PJ, Fawcett JP, Lin DC *et al.* A polarity complex of mPar-6 and atypical PKC binds, phosphorylates and regulates mammalian Lgl. *Nat Cell Biol* 2003; **5**: 301–308.
77. Yamanaka T, Horikoshi Y, Sugiyama Y *et al.* Mammalian Lgl forms a protein complex with PAR-6 and aPKC independently of PAR-3 to regulate epithelial cell polarity. *Curr Biol* 2003; **13**: 734–743.
78. Hong Y, Ackerman L, Jan LY, Jan YN. Distinct roles of Bazooka and Stardust in the specification of *Drosophila* photoreceptor membrane architecture. *Proc Natl Acad Sci USA* 2003; **100**: 12712–12717.
79. Penkert RR, DiVittorio HM, Prehoda KE. Internal recognition through PDZ domain plasticity in the Par-6-Pals1 complex. *Nat Struct Mol Biol* 2004; **11**: 1122–1127.
80. Kempkens O, Medina E, Fernandez-Ballester G *et al.* Computer modelling in combination with *in vitro* studies reveals similar binding affinities of *Drosophila* Crumbs for the PDZ domains of Stardust and DmPar-6. *Eur J Cell Biol* 2006; **85**: 753–767.
81. Sotillos S, Diaz-Meco MT, Caminero E *et al.* DaPKC-dependent phosphorylation of Crumbs is required for epithelial cell polarity in *Drosophila*. *J Cell Biol* 2004; **166**: 549–557.
82. Yeaman C, Grindstaff KK, Nelson WJ. New perspectives on mechanisms involved in generating epithelial cell polarity. *Physiol Rev* 1999; **79**: 73–98.
83. Nelson WJ. Adaptation of core mechanisms to generate cell polarity. *Nature* 2003; **422**: 766–774.
84. Yin HL, Janmey PA. Phosphoinositide regulation of the actin cytoskeleton. *Annu Rev Physiol* 2003; **65**: 761–789.
85. Janetopoulos C, Devreotes P. Phosphoinositide signaling plays a key role in cytokinesis. *J Cell Biol* 2006; **174**: 485–490.
86. Shi SH, Jan LY, Jan YN. Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity. *Cell* 2003; **112**: 63–75.
87. Nakanishi H, Brewer KA, Exton JH. Activation of the zeta isozyme of protein kinase C by phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem* 1993; **268**: 13–16.
88. Le Good JA, Ziegler WH, Parekh DB *et al.* Protein kinase C isotypes controlled by phosphoinositide 3-kinase through the protein kinase PDK1. *Science* 1998; **281**: 2042–2045.
89. Pinal N, Goberdhan DC, Collinson L *et al.* Regulated and polarized PtdIns(3,4,5)P3 accumulation is essential for apical membrane morphogenesis in photoreceptor epithelial cells. *Curr Biol* 2006; **16**: 140–149.
90. Gassama-Diagne A, Yu W, ter Beest M *et al.* Phosphatidylinositol-3,4,5-trisphosphate regulates the formation of the basolateral plasma membrane in epithelial cells. *Nat Cell Biol* 2006; **8**: 963–970.
91. Martin-Belmonte F, Gassama A, Datta A *et al.* PTEN-mediated apical segregation of phosphoinositides controls epithelial morphogenesis through Cdc42. *Cell* 2007; **128**: 383–397.
92. Erasmus JC, Braga VM. Rho GTPase activation by cell–cell adhesion. *Methods Enzymol* 2006; **406**: 402–415.
93. Braga VM. Cell–cell adhesion and signalling. *Curr Opin Cell Biol* 2002; **14**: 546–556.
94. Pruyne D, Legesse-Miller A, Gao L *et al.* Mechanisms of polarized growth and organelle segregation in yeast. *Annu Rev Cell Dev Biol* 2004; **20**: 559–591.
95. Garcia P, Tajadura V, Garcia I, Sanchez Y. Role of Rho GTPases and Rho-GEFs in the regulation of cell shape and integrity in fission yeast. *Yeast* 2006; **23**: 1031–1043.
96. Nakagawa M, Fukata M, Yamaga M *et al.* Recruitment and activation of Rac1 by the formation of E-cadherin-mediated cell–cell adhesion sites. *J Cell Sci* 2001; **114**: 1829–1838.
97. Fukata M, Nakagawa M, Kaibuchi K. Roles of Rho-family GTPases in cell polarisation and directional migration. *Curr Opin Cell Biol* 2003; **15**: 590–597.
98. Liu XF, Ishida H, Raziuddin R, Miki T. Nucleotide exchange factor ECT2 interacts with the polarity protein complex Par6/Par3/protein kinase Czeta (PKCzeta) and regulates PKCzeta activity. *Mol Cell Biol* 2004; **24**: 6665–6675.
99. Chen X, Macara IG. Par-3 controls tight junction assembly through the Rac exchange factor Tiam1. *Nat Cell Biol* 2005; **7**: 262–269.
100. Mertens AE, Rygiel TP, Olivo C *et al.* The Rac activator Tiam1 controls tight junction biogenesis in keratinocytes through binding to and activation of the Par polarity complex. *J Cell Biol* 2005; **170**: 1029–1037.
101. Nishimura T, Yamaguchi T, Kato K *et al.* PAR-6–PAR-3 mediates Cdc42-induced Rac activation through the Rac GEFs STEF/Tiam1. *Nat Cell Biol* 2005; **7**: 270–277.
102. Malliri A, van Es S, Huveneers S, Collard JG. The Rac exchange factor Tiam1 is required for the establishment and maintenance of cadherin-based adhesions. *J Biol Chem* 2004; **279**: 30092–30098.
103. Chen X, Macara IG. Par-3 mediates the inhibition of LIM kinase 2 to regulate cofilin phosphorylation and tight junction assembly. *J Cell Biol* 2006; **172**: 671–678.
104. Macara IG, Spang A. Closing the GAP between polarity and vesicle transport. *Cell* 2006; **125**: 419–421.
105. Aijaz S, D'Atri F, Citi S *et al.* Binding of GEF-H1 to the tight junction-associated adaptor cingulin results in inhibition of Rho signaling and G1/S phase transition. *Dev Cell* 2005; **8**: 777–786.
106. Otani T, Ichii T, Aono S, Takeichi M. Cdc42 GEF Tuba regulates the junctional configuration of simple epithelial cells. *J Cell Biol* 2006; **175**: 135–146.
107. Baas AF, Kuipers J, van der Wel NN *et al.* Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. *Cell* 2004; **116**: 457–466.
108. Tepass U, Tanentzapf G, Ward R, Fehon R. Epithelial cell polarity and cell junctions in *Drosophila*. *Annu Rev Genet* 2001; **35**: 747–784.
109. Harris TJ, Peifer M. Adherens junction-dependent and -independent steps in the establishment of epithelial cell polarity in *Drosophila*. *J Cell Biol* 2004; **167**: 135–147.
110. Harris TJ, Peifer M. The positioning and segregation of apical cues during epithelial polarity establishment in *Drosophila*. *J Cell Biol* 2005; **170**: 813–823.
111. Bose R, Wrana JL. Regulation of Par6 by extracellular signals. *Curr Opin Cell Biol* 2006; **18**: 206–212.
112. Dressler GR. The cellular basis of kidney development. *Annu Rev Cell Dev Biol* 2006; **22**: 509–529.
113. Thiery JP. Epithelial–mesenchymal transitions in tumour progression. *Nat Rev Cancer* 2002; **2**: 442–454.
114. Ozdamar B, Bose R, Barrios-Rodiles M *et al.* Regulation of the polarity protein Par6 by TGFbeta receptors controls epithelial cell plasticity. *Science* 2005; **307**: 1603–1609.

115. Barrios-Rodiles M, Brown KR, Ozdamar B *et al.* High-throughput mapping of a dynamic signaling network in mammalian cells. *Science* 2005; **307**: 1621–1625.
116. Vaezi A, Bauer C, Vasioukhin V, Fuchs E. Actin cable dynamics and Rho/Rock orchestrate a polarized cytoskeletal architecture in the early steps of assembling a stratified epithelium. *Dev Cell* 2002; **3**: 367–381.
117. Sahai E, Marshall CJ. ROCK and Dia have opposing effects on adherens junctions downstream of Rho. *Nat Cell Biol* 2002; **4**: 408–415.
118. Barrallo-Gimeno A, Nieto MA. The Snail genes as inducers of cell movement and survival: implications in development and cancer. *Development* 2005; **132**: 3151–3161.
119. Aigner K, Dampier B, Descovich L *et al.* The transcription factor ZEB1 (deltaEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity. *Oncogene* 2007; advance online publication, 7 May 2007.